DATA EVALUATION RECORD

STUDY TYPE: POSITIVE CONTROL – DEVELOPMETNAL NEUROTOXICITY STUDY IN WISTAR RATS BY BASF CORPORATION
MRID 46210605

Prepared for

Health Effects Division, Office of Pesticide Programs
U.S. Environmental Protection Agency
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DATA EVALUATION RECORD

STUDY TYPE: Positive Control Developmental Neurotoxicity Study - Rat; OPPTS 870.6300 (' 83-6); OECD 426 (draft)

PC CODE: N/A **DP BARCODE:** N/A

TEST MATERIAL (PURITY): MAM (Methylazoxy methanol acetate) (>96% a.i.)

SYNONYMS: Methyl-O,N,N-azoxymethanol acetate

CITATION: Kaufmann, W., K., Schneider, and B. van Ravenzwaay, (2003) Methylazoxy methanol acetate. Positive control - Developmental neurotoxicity study in Wistar rats. Single intraperitoneal administration to the dams. BASF. Aktiengesellschaft, D-67056 Ludwigshafen/Rhein, Germany. Laboratory project identification no. 03R0076/02004, November 11, 2003. MRID 46210605. Unpublished

SPONSOR: None.

EXECUTIVE SUMMARY:

In a developmental neurotoxicity study (MRID 46210605), MAM (>96% a.i.; batch # ET11-109-1) was administered to 42 presumed-pregnant female Wistar rats per dose by a single intraperitoneal administration at dose levels of 0, 7.5, 15, or 30 mg/kg bw on gestation day (GD) 15. An open field observation was performed on 10 dams/group on GDs 7 and 16 and on lactation days 7 and 14. On postnatal day 4, litters were culled to yield 8 pups/litter (assumed 4/sex/litter, but not stated). Offspring representing 20 litters/dose were allocated for detailed open field observation, assessment of motor activity, assessment of auditory startle response habituation, and assessment of learning and memory. On PNDs 11, 22, and 62, the whole brain was collected from 10 pups/sex/dose for micropathological examination and morphometric analysis. These pups were also examined for neuropathology.

Treatment-related and dose-dependent effects noted in the offspring included: gross lesions at the mid- and high-dose (hypoplasia of the cortex, cerebri), neurohistopathological lesions at the high-dose (disorganization of the cortical layers, clusters of ectopic hippocampal and periventricular neurons, and ventricle dilation), decrements in brain weight compared to controls (absolute and relative brain weights; low-, mid- and high-dose males; mid- and high-dose females), and a dose-related reduction in morphometric measurements (greatest reductions occurring in the corpus callosum, the hippocampus, the parietal and the frontal cortices; low-, mid, and/or high-dose groups affected). Basal ganglia (nucleus caudatus/putamen) and the cerebellum (folium

pyramis, external germinal matrix layer) also showed reduced size, indicating a treatment-related influence on the development of the brain. Corresponding with the morphological abnormalities, increased motor activity, impaired auditory habituation, and retarded learning/relearning capability were noted at the mid- and high-dose. The neurotoxic effects did not become apparent until the affected offspring had developed to young adults (PND 60). Females appeared to be more sensitive.

Effects in the maternal rats were limited to reduced body weight gain (over GDs 15-20 and 1-20) and feed consumption (over GDs 15-20 and lactation days 1-7).

The Experimental Toxicology and Ecology Facility of the BASF Corporation has demonstrated proficiency in testing for developmental neurotoxicity in rats through open field observation, motor activity assessment, auditory startle reflex habituation, learning and memory testing (water maze performance), brain weight, gross and miscroscopic pathological findings in the brain, and morphometric brain measurements.

This positive control study for developmental neurotoxicity in rats is classified as **Acceptable/Non guideline**. Although analytical determination of the stability, homogeneity and concentration of the test formulations was not performed, the deviations did not affect the acceptability of the study. The study was designed as a positive control study to demonstrate that the laboratory is proficient in performing developmental neurotoxicity tests.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** MAM

Description:	Liquid/colorless
Lot/batch #:	ET11-109-1
Purity:	>96% a.i.
Compound stability:	Not provided; stated that reanalysis not necessary
CAS # of TGAI:	592-62-1
Structure:	Not available

2. **Vehicle and/or positive control:** physiologic saline solution

3. **Test animals (P):**

Species:	rats
Strain:	Wistar (CrlGlxBrlHan:WI)
Age at study initiation:	10-12 wks
Wt. at study initiation:	139.5-186.3 g
Source:	Charles River Laboratories, Germany
Housing:	Individually in stainless steel wire mesh cages (except from GD 18 until day 21 after birth, when rats housed in Makrolon type M III cages)
Diet:	Kliba maintenance diet rat/mouse/hamster meal (Provimi Kliba SA, Kaiseraugst, Switzerland) *ad libitum*
Water:	Tap water *ad libitum*
Environmental conditions:	Temperature: 20-24EC Humidity: 30-70% Air changes: Information not provided Photoperiod: 12 hrs dark/ 12 hrs light
Acclimation period:	Animals were acclimated to laboratory conditions between the start of the study (GD 0) and the day of test substance administration (GD 15).

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates:** Start: March 11, 2002; End: June 11, 2002

2. **Study schedule:** The maternal animals were timed mated by the breeder and supplied on presumed gestation (GD) 0. The test substance was administered to the maternal animals once on GD 15. After parturition, only those litters which consisted of at least 8 pups and whose date of littering (day 0) was on 4 consecutive days were used for further examinations. All other litters and dams without a litter were sacrificed and discarded without further examination. Pups retained for the study were weaned on PND 21, after which time maternal animals were killed. F_1 pups remained on study up until PND 62 (study termination).

3. **Mating procedure:** The maternal animals were timed mated by the breeder. The day that sperm or a vaginal plug was detected was designated as gestation day (GD) 0. The animals were supplied to the testing laboratory on the same day. Each pregnant female was housed individually in a stainless steel wire mesh cage, except from GD 18 until day 21 after birth,

when the dams, their litters, and subset II rats were housed in Makrolon type M III cages. Dams were provided with nesting material toward the end of gestation.

4. **Animal assignment**: Mated females were assigned to dose groups as indicated in Table 1 (method of assignment not provided). The dams were allowed to litter and rear their pups until lactation day (LD) 4 or 21.

   The individual litters were standardized on PND 4 such that each litter contained at least 8 pups. All litters that consisted of fewer than 8 pups were not used for the examinations and the pups and dams were sacrificed and discarded without any further examination. Of the litters remaining, one male or one female pup/litter were selected from each litter (10 pups/sex/group) before the beginning of the examinations and were allocated to the specific subsets (I-VI) (Table 1).

TABLE 1. Study design

Experimental parameter		Dose (mg/kg)			
		0	7.5	15	30
Maternal animals					
No. of maternal animals assigned		41	42	42	42
Open Field Observation (GD 7, 16, LD 7,14)		10	10	10	10
Offspring					
Subset I	Perfusion fixation, brain wt, neuropathology (PND 11)	10/sex	10/sex	10/sex	10/sex
Subset II	Perfusion fixation, brain wt, neuropathology (PND 22)	10/sex	10/sex	10/sex	10/sex
Subset III	Auditory startle test (PND 24, 60) Perfusion fixation, brain wt, neuropathology (PND 62)	10/sex	10/sex	10/sex	10/sex
Subset IV	Open field observation (PND 4, 11, 21, 35, 45, and 60) Motor activity (PND 13, 17, 21, and 60)	10/sex	10/sex	10/sex	10/sex
Subset V	Learning and memory test (PND 23)	10/sex	10/sex	10/sex	10/sex
Subset VI	Learning and memory test (PND 60)	10/sex	10/sex	10/sex	10/sex

5. **Dose selection rationale:** It was not stated how the dose levels were chosen, other than that the 7.5 mg/kg bw dose was the expected NOAEL.

6. **Dosage administration:** All doses were administered once to maternal animals intraperitoneally on GD 15 in a volume of 5 mL/kg of body weight/day. Dosing was based on GD 15 body weight.

7. **Dosage preparation and analysis:** Formulations were prepared once on the day of administration by mixing appropriate amounts of test substance with physiological saline solution and thoroughly mixing with a magnetic stirrer. Appropriate formulations were prepared by dilution. Analyses for concentration, homogeneity, and stability of the test substance in physiological saline solution were not evaluated

Results:

Homogeneity analysis: Not conducted.

Stability analysis: Not conducted

Concentration analysis: Not conducted.

Analytical data were not available to determine if the mixing procedure was adequate or if the difference between nominal and actual dosage to the study animals was acceptable.

C. OBSERVATIONS:

1. In-life observations:

a. Maternal animals: Mortality or moribundity in dams was checked twice daily on working days and once daily during the weekends or on holidays. Clinical signs of toxicity were recorded daily.

Ten dams per group were observed outside the home cage for open field observations (OFO) twice during gestation (GDs 7 and 16) and twice during the lactation period (LDs 7 and 14). The examinations were started in the morning. The findings were graded according to their intensity on the basis of an index of findings or were described in detail. For the observation, the dams were removed from their cages and placed in a standard arena (50 x 37.5 cm with a lateral border of 25 cm). The following open field observations were recorded.

X	FUNCTIONAL OBSERVATIONS
X	Signs of autonomic function, including: 1) Ranking of degree of lacrimation and salivation, with range of severity scores from none to severe 2) Presence or absence of piloerection (observation of skin) and exophthalamus (eye size), 3) Ranking or count of urination and defecation 4) Pupillary function such as measure of pupil size 5) Degree of palpebral closure.
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements/stereotypics.
X	Description and incidence of posture and gait abnormalities.
X	Description of behavior in handling when removed from the cage, appearance of fur, nasal discharge, respiration, activity/arousal level, and any other observations

Further details were not provided, such as whether the same technicians were used throughout testing, if the technicians were blind to treatment status of animals, description of the environmental conditions (e.g., noise level, etc.), and duration of testing.

Individual maternal body weight and feed consumption data were recorded on GD 0, 6, 13, 15, and 20, and on LD days 1, 7, 14, and 20 (except feed consumption not measured on LD 20).

After pups were weaned on PND 21, the dams were killed and discarded without further examination. Dams without a litter were discarded after the uterus had been stained for the evidence of early resorptions.

b. Offspring:

1) **Litter observations:** The sex and number of all delivered pups were determined as soon as possible on the day of birth, and pups were examined for external findings. Litters were examined twice daily for dead or moribund pups (once daily on weekends and holidays).

   On PND 4, litters were standardized to a maximum of 8 pups/litter (assume 4/sex/litter, but not stated). Litters that consisted of fewer than 8 pups were removed from the study.

   The pups were weighed on the day after birth (PND 1), PND 4 (before standardization), and PNDs 11, 17, and 21.

2) **Developmental landmarks**: Beginning on PND 40, male offspring were examined daily for balanopreputial separation. Beginning on PND 27, female offspring were examined daily for vaginal patency. The age of onset of sexual maturation was recorded and the body weight determined.

3) **Postweaning observations:** After weaning on PND 21, offspring were examined twice daily for mortality, and cage-side observations were conducted once daily. Individual offspring body weight data were recorded weekly.

4) **Neurobehavioral evaluation**: Observations and the schedule for those observations are summarized as follows from the report:

i. **Detailed open field observation (OFO):** On PNDs 4, 11, 21, 35, 45, and 60, a total of 10 offspring/sex/group (one male or one female from each litter) was examined outside the home cage in an open arena, as appropriate for the developmental stage being observed. The same parameters assessed in the maternal OFO were examined for offspring. The OFO was carried out in the morning.

ii. **Motor activity testing:** Motor activity was evaluated in 10 rats/sex/dose (Subset IV animals) on PNDs 13, 17, 21, and 60 using the Tru Scan Photobeam Linc. The animals were measured in a darkened room in one of ten enclosures equipped with two sensor rings each with 16 beams per cage side. For each rat, the distance covered and the number of rearings was measured over 12 intervals, each lasting five minutes, for a total of 1 hour. The examinations were generally conducted in the afternoon (~1 p.m.)

iii. **Auditory startle reflex habituation:** Auditory startle reflex habituation testing was performed on 10 offspring/sex/dose on postnatal days 24 and 60 (Subset III animals) using an automated system (SR-Lab, Startle Response System; San Diego Instruments). The animals were given a 5 minute acclimation period in the response chamber with a 70 dBA background noise. The startle response was recorded in 50 identical trials at a startle stimulus sound level of 120 dBA with a 5 second interval between the trials. Response was recorded for 50 milliseconds. Measurements were carried out with the light and ventilator switch on in the measurement chambers. Data, such as maximum amplitude and latency to the peak of the response, were

analyzed in 5 blocks of 10 trials each. The examinations were generally conducted in the afternoon.

iv. **Learning and memory testing:** Learning and memory testing was performed in 10 offspring/sex/dose on PNDs 23 and 30 (Subset V animals) and a different 10 offspring/sex/dose on PNDs 60 and 67 (Subset VI animals) using the water maze test. The water maze test consisted of 3 parts and was performed within 2 weeks, starting with learning ability in the first week, followed by memory and relearning ability in the second week. Learning ability testing (learning 1) consisted of 6 trials at intervals of 1 hour for each selected animal. At every trial, the animals had to find a ladder (escape) on the right side of the M-shape water maze pool. The animals had a maximum duration of 6 minutes to swim for each trial. If an animal found the straight way to escape, it was scored positive (+). If the animals went a wrong way, it was scored negative (-), but it was left in the water until it either found the escape or time was up. Memory was tested one week later when the same animals had to find the ladder (escape) on the right side of the water maze pool again. The relearning ability testing (learning 2) started an hour after completion of the memory test. The same experimental procedure was followed as in the learning 1 test, except that the ladder (escape) was placed at the left side of the M-shape water maze pool. The initial trial results from learning 1 or learning 2 testing were not included in the evaluation since they served as acclimation trials in the test.

5) **Cholinesterase determination:** Cholinesterase activity was not determined.

6) **Pharmacokinetic data:** Pharmacokinetic data were not collected.

2. **Postmortem observations:**

a. **Maternal animals:** Maternal animals were sacrificed by cervical dislocation after the pups had been weaned (LD 21) and discarded without postmortem examination. Animals without a litter were also discarded after the uterus had been stained for evidence of early resorptions.

b. **Offspring**: The offspring selected for brain weight or neuropathological evaluation (Subset I, II, and III) were sacrificed on PND 11, 22, or 60. These animals were subjected to postmortem examinations as described below.

On postnatal day 11, 22, or 60, 10 animals/sex/group (one male or one female per litter) were anesthetised and sacrificed by perfusion fixation (type of perfusant not specified). Brains (with olfactory bulbs) from these animals were weighed after removal but before any further preparation. The brain length (on a line extending from the rostral end of the frontal lobe to the caudal medulla oblongata of the cerebellum) and maximum width (pituitary region) were measured. From all of these 10 animals/sex in the control and high-dose group on PNDs 11, 22, and 60, the central nervous tissues (marked with an X in the following table) were dissected, embedded in paraplast, sectioned, and stained with hematoxylin-eosin. From all of these 10 animals/sex in the control and high-dose group only on PND 60, the peripheral nervous tissues (marked with an X in the following table)

were dissected, subjected to secondary fixation in 5% glutaraldehyde solution, embedded in plastic (epoxy resin), sectioned semi-thinly, and stained with Azure II-Methylene blue-basic Fuchsin. Of the central nervous system tissues from rats in the low- and mid-dose groups, the brain cross sections were embedded in paraplast, while the remaining central nervous system tissues were preserved in neutrally buffered, 4% formaldehyde solution. Peripheral nervous system tissues from rats in the low- and mid-dose groups were subjected to secondary fixation in 5% glutaraldehyde solution and stored in buffer solution. In summary, histopathological examination of the central nervous system tissues was performed on tissues from control and high-dose pups sacrificed on PNDs 11, 22, and 60, while histopathological examination of the peripheral nervous system tissues was performed only on tissues from control and high-dose pups sacrificed on PND 60.

The CHECKED (X) tissues were evaluated for adult offspring.

X	CENTRAL NERVOUS SYSTEM (PND 11, 22, 60 pups)	X	PERIPHERAL NERVOUS SYSTEM (only PND 60 pups)
	BRAIN		**SCIATIC NERVE**
X	Olfactory bulb	X	Proximal sciatic nerve
X	Frontal lobe		
X	Parietal lobe with diencephalon		
X	Midbrain with occipital and temporal lobe		
X	Cerebellum (2 planes of section)	**X**	**OTHER**
X	Pons	X	Proximal tibial nerve (at knee)
X	Medulla oblongata	X	Distal tibial nerve (at lower leg)
X	**SPINAL CORD** (longitudinal sections; ***cross sections***)	X	Lumbar dorsal root fibers
X	Cervical swelling I (C1-C3; ***C1***)	X	Lumbar dorsal root ganglion
X	Cervical swelling II (C3-C5; ***C5***)	X	Lumbar ventral root fibers
X	Thoracic cord (T5-8; ***T8***)	X	Cervical dorsal root ganglion
X	Lumbar swelling	X	Cervical dorsal root fibers
X	**OTHER**	X	Cervical ventral root fibers
X	Gasserian ganglia with nerve		
X	Gastronemius muscle (longitudinal and cross sections)		
X	Eyes with retinal and optical nerve		

Morphometry of the brain areas was performed on the same animals selected for neuropathology. Detailed morphometric evaluation consisted of:

- the neocortex (width of the total cortical mantle [layers I – VI from the surface of the pia matter to the white substance] was measured vertically to a tangent over a region of the frontal and parietal cortices determined prior);
- the caudate nucleus/putamen (the largest lateral extension of the left and the right part was determined);
- the corpus callosum (the width was measured at the middle line of the cross section), hippocampus (the largest dorsoventral extension was measured);
- and the cerebellum (the width of a select folium [e.g. folium pyramis] was measured in the middle of a line which runs vertically to a tangent from the tip to the base of the folium)

For thickness measurements of these brain layers, measurements were carried out on the left and right brain with the exception of the corpus callosum and cerebellum.

D. DATA ANALYSIS:

1. **Statistical analyses:** Feed consumption (females), body weight and body weight change (females and pups; for the pups weight, the litter means were used), duration of gestation, and the number of pups delivered per litter were analyzed using simulatenous comparison of all dose groups with the control group using the Dunnett-test (two-sided) for the hypothesis of equal means. Female fertility index, gestation index, females with liveborn pups, females with stillborn pups, females with all stillborn pups, live birth index, pups stillborn, pups died, pups cannibalized, pups sacrificed moribund, viability index, lactation index, and water maze evaluation were evaluated using a pairwise comparison of each dose group with the control group using Fisher's exact test for the hypothesis of equal proportions. The water maze evaluation was also analyzed using a pairwise comparison of each dose group with the control group using the Wilcoxon-test (one-sided) for the hypothesis of equal medians. Motor activity and startle response were evaluated using non-parametric one-way analysis using Kruskal-Wallis test (two-sided). If the resulting p-value was equal to or less than 0.05, a pairwise comparison of the dose groups with the control group was performed using Mann-Whitney U-test (two-sided) for the hypothesis of equal medians. Weight parameters recorded during neuropathology were evaluated using a non-parametric one-way analysis using Kruskal-Wallis test (two-sided). If the resulting p-value was equal to or less than 0.05, a pairwise comparison of each dose group with the control group was performed using Wilcoxon-test (two-sided) for the hypothesis of equal medians. Morphometric parameters were analyzed using a pairwise comparison of each dose group with the control group using the Wilcoxon-test (one-sided) with Bonferoni-Holm-Adjustment for the hypothesis of equal medians.

   Levels of significance were set at $p \leq 0.05$ and $p \leq 0.01$.

2. **Indices:**

   a. **Reproductive indices:** The following reproductive indices were calculated from breeding and parturition records of animals in the study:

   $$\text{Female fertility index (\%)} = \frac{\text{number of females pregnant}}{\text{Number of females mated}} \times 100$$

   $$\text{Gestation index (\%)} = \frac{\text{number of females with live pups on the day of birth}}{\text{Number of females pregnant}} \times 100$$

   where:

   number of females pregnant is defined as the number of females that gave birth to a litter or with pups/fetuses in utero

   number of females mated is defined as the number of females with vaginal sperm or that gave birth to al litter or with fetuses in utero

   b. **Offspring viability indices:** Indices of offspring survival were not calculated.

3. **Positive and historical control data:** Historical control data were not included in this study. The purpose of the study was to generate positive control data for developmental neurotoxicity testing.

II. RESULTS:

A. PARENTAL ANIMALS:

1. **Mortality and clinical and functional observations:** No mortalities occurred in any of the dose groups, and no substance-related clinical findings were observed in the dams during gestation or lactation (clinical findings occurred in individual animals and were not related to dose). One dam in the control group aborted. No treatment-related findings were recorded during open field observations.

2. **Body weight and food consumption:** Selected group mean body weight and food consumption values for pregnant or nursing dams are summarized in Table 2.

   During gestation, no statistically significant differences in mean maternal body weight were noted, but body weight gain was statistically reduced in the high-dose group over GDs 15-20 and 0-20. No effects on mean maternal body weight or body weight gain were noted during lactation.

   Mean feed consumption (g/animal/day) was statistically decreased in high-dose dams during GDs 15-20, over the entire gestational period of 0-20, and over LDs 1-7. No other statistically or biologically significant effects on feed consumption were recorded.

TABLE 2. Mean (∀SD) maternal body weight and food consumption [a]

Observations/study week	Dose (mg/kg)			
	0	7.5	15	30
Gestation [b]				
Mean body weight (g)				
Gestation day 0	155.3 ± 8.6	159.3 ± 11.3	158.1 ± 11.3	157.5 ± 9.3
Gestation day 6	184.9 ± 10.6	188.6 ± 12.0	188.3 ± 11.8	187.6 ± 10.5
Gestation day 15	226.5 ± 14.8	230.10 ± 15.1	230.9 ± 13.0	230.3 ± 12.7
Gestation day 20	270.9 ± 22.7	271.3 ± 23.6	271.0 ± 20.9	259.9 ± 15.1
Mean weight gain (g)				
Gestation days 0-6	29.5 ± 5.0	29.4 ± 5.1	30.3 ± 3.6	30.1 ± 4.1
Gestation days 6-13	31.0 ± 5.7	30.7 ± 6.1	32.6 ± 6.6	32.2 ± 5.5
Gestation days 15-20	44.4 ± 15.0	41.2 ± 15.2	40.1 ± 16.5	29.6** ± 6.21 (-33) [c]
Gestation days 0-20	115.6 ± 19.5	112.1 ± 20.6	113.0 ± 19.3	102.4** ± 12.2 (-11)
Mean food consumption (g/animal/day)				
Gestation days 0-6	17.0 ± 1.7	16.9 ± 1.6	17.2 ± 1.4	17.4 ± 1.3
Gestation days 6-13	19.9 ± 1.9	20.1 ± 1.8	20.4 ± 1.6	20.5 ± 1.7
Gestation days 15-20	20.8 ± 2.8	20.5 ± 3.1	20.6 ± 3.1	16.7** ±1.6 (-20)
Lactation [d]				
Mean body weight (g)				
Lactation day 1	217.1 ± 13.3	217.4 ± 15.9	216.2 ± 15.6	211.6 ± 11.7
Lactation day 7	236.2 ± 14.2	234.4 ± 15.9	235.6 ± 16.3	229.0 ± 11.6
Lactation day 14	255.9 ± 18.5	252.5 ± 15.4	255.2 ± 16.8	249.2 ± 13.5
Lactation day 21	253.8 ± 17.1	249.2 ± 16.3	250.8 ± 13.4	246.7 ± 14.2
Mean weight gain (g)				
Lactation days 1-21	36.7 ± 11.1	31.8 ± 7.1	34.5 ± 10.3	35.1 ± 10.9
Mean food consumption (g/animal/day)				
Lactation days 1-7	31.9 ± 2.6	30.4 ± 2.8	31.3 ± 3.0	29.2** ± 3.0 (-8)
Lactation days 7-14	46.3 ± 4.4	45.2 ± 3.6	47.6 ± 4.2	45.1 ± 3.5

[a] Data obtained from pages 108-113, MRID 46210605.

[b] N =37, 34, 35, and 34 for the control, low-, mid-, and high-dose groups, respectively

[c] Number in parentheses is the percent increase or decrease relative to controls; calculated by reviewer

[d] N =26, 24, 25, and 22 for the control, low-, mid-, and high-dose groups, respectively

* Statistically different from control, $p<0.05$.

** Statistically different from control, $p<0.01$.

3. **Test substance intake:** The test substance was administered by a single intraperitoneal injection at a dose of 0, 7.5, 15, or 30 mg/kg bw.

4. **Reproductive performance:** Results for the maternal animals are summarized in Table 3. No treatment-related effects on reproductive performance were noted.

TABLE 3. Reproductive performance [a]				
Observation	Dose (mg/kg)			
	0	7.5	15	30
Number mated	41	42	42	42
Number of litters	39	40	39	37
Intercurrent deaths	0	0	0	0
Mean (∀SD) gestation duration (days)	21.6 ± 0.60	21.6 ± 0.59	21.6 ± 0.49	21.5 ± 0.56
Female mating index (%)	100	100	100	100
Female fertility index (%)	98	100	95	88
Gestation index (%)	98	95	98	100

[a] Data obtained from page 114, MRID 46210605.

5. **Maternal postmortem results**: Maternal animals were not subjected to gross necropsy; therefore, no postmortem findings were recorded.

B. OFFSPRING:

1. **Viability and clinical signs**: Litter size and viability results from pups during lactation are summarized in Table 4. No dose-related effects of treatment were noted in litter size, sex ratio, or pup viability. The lactation and viability indices were not provided. Litter size data are as reported in the study. However, the reviewer could not confirm mean litter size from the individual animal data. Standardization was supposed to be to 8 pups/litter but a review of the individual litter data could not confirm the mean litter size reported in the summary table. Pups removed on day 11 for neuropathology account for the decrease between days 11 and 17.

   No dose-related effects were noted during clinical observations of offspring.

TABLE 4. Litter size and viability [a]

Observation	Dose (mg/kg)			
	0	7.5	15	30
Total number born	358	355	336	302
Number born live	353	355	336	300
Number born dead	5	0	0	2
Sex Ratio Day 0 (% %)	42.8	50.7	53.9	48.0
# Deaths Days 1-4 (%)	2.8	1.4	2.4	1.0
# Deaths Days 4-21 (%)	0	0	0	~0.3%
Mean litter size:				
Day 0	9.1 ± 1.8	8.9 ± 1.4	8.6 ± 2.0	8.1 ± 2.5
Day 4 [b]	6.4 ± 4.7	5.5 ± 4.6	5.9 ± 4.6	5.4 ± 4.7
Day 4 [c]	5.3 ± 3.8	4.8 ± 4.0	5.1 ± 3.9	4.8 ± 4.0
Day 11	5.3 ± 3.8	4.8 ± 4.0	5.1 ± 3.9	4.7 ± 4.0
Day 17	4.8 ± 3.5	4.3 ± 3.6	4.6 ± 3.5	4.2 ± 3.5
Day 21	4.8 ± 3.5	4.3 ± 3.6	4.6 ± 3.5	4.2 ± 3.5
Live birth index (%)	99	100	100	99
Viability index	Not calculated	Not calculated	Not calculated	Not calculated
Lactation index	Not calculated	Not calculated	Not calculated	Not calculated

[a] Data obtained from pages 114-117 in the study report.

[b] Before standardization (culling).

[c] After standardization (culling).

* Statistically different from control, $p<0.05$

** Statistically different from control, $p<0.01$

2. **Body weight**: Male and female offspring from the high-dose group had statistically significant decreases in mean weight at all time points during lactation compared to controls. Mean pup body weight change was decreased in male and female offspring during lactation days 1-4, but was comparable to controls thereafter. Selected mean preweaning pup body weight data are presented in Table 5.

TABLE 5. Mean (∀SD) pre-weaning pup body weight (g) [a]

Postnatal day	Dose (mg/kg)							
	0	7.5	15	30	0	7.5	15	30
	Males				Females			
1	6.4 ± 0.6	6.5 ± 0.7	6.4 ± 0.7	5.6** ± 0.6	6.1 ± 0.6	6.0 ± 0.7	6.0 ± 0.7	5.3** ± 0.6
4 b	9.8 ± 1.0	9.8 ± 1.0	9.8 ± 1.0	8.4** ± 1.0	9.4 ± 1.0	9.3 ± 1.1	9.3 ± 1.0	8.1** ± 1.0
4 c	9.8 ± 1.0	9.9 ± 1.0	9.8 ± 1.0	8.4** ± 1.0	9.4 ± 1.0	9.3 ± 1.1	9.3 ± 1.0	8.1** ± 1.0
11	22.3 ± 2.0	22.0 ± 2.0	22.2 ± 2.1	20.0** ± 2.1	21.5 ± 2.0	21.1 ± 2.3	21.4 ± 2.1	19.3** ± 2.2
17	36.4 ± 2.9	35.5 ± 2.6	36.6 ± 3.4	33.7* ± 3.1	34.9 ± 2.8	34.1 ± 2.9	35.2 ± 3.2	32.8* ± 3.0
21	47.8 ± 4.3	46.9 ± 3.5	48.2 ± 4.1	44.4* ± 4.5	45.8 ± 3.5	45.0 ± 3.9	46.2 ± 3.8	42.8* ± 4.2
Body wt gain: 1-4	3.4 ± 0.5	3.3 ± 0.4	3.4 ± 0.5	2.8** ± 0.5	3.3 ± 0.5	3.2 ± 0.5	3.3 ± 0.5	2.8** ± 0.5
Body wt gain: 4-21	38.0 ± 3.6	37.0 ± 3.0	38.4 ± 3.5	36.0 ± 3.9	36.4 ± 2.8	35.7 ± 3.1	36.9 ± 3.0	34.7 ± 3.5

[a] Data obtained from pages 118-121, MRID 46210605.
[b] Before standardization (culling).
[c] After standardization (culling).
* Statistically different from control, $p<0.05$
** Statistically different from control, $p<0.01$

Selected mean post weaning offspring body weight data are presented in Table 6. Body weight decrements were not consistent among the groups. Subsession III male offspring from the high-dose group had statistically significant decreases in mean body weight during post weaning weeks 3, 4, and 5 (11, 12, and 11% decrease relative to controls, respectively) and mean body weight gain during post weaning weeks 0-1 (17% decrease), 2-3 (14% decrease), and weeks 0-5 (12% decrease). Male offspring from the low-dose group had statistically significant decreases in mean body weight gain during post weaning weeks 4-5 (18% decrease) and 0-5 (12% decrease). Subsession IV male offspring from the high-dose group had a statistically significant decrease in mean body weight gain during post weaning weeks 0-1 (15% decrease). Subsession V male offspring in the high-dose group had statistically significant decreases in mean body weight at post weaning week 1 (14% decrease) and body weight gain during post weaning weeks 0-1 (19% decrease). No statistically significant decreases in mean body weight or body weight gain were noted in any groups of female offspring.

TABLE 6. Mean (∀SD) post-weaning pup body weights (g) [a]

Week after weaning [b]	Dose (mg/kg)							
	0	7.5	15	30	0	7.5	15	30
	Males				Females			
Subset III								
Body weight								
Week 0	50 ± 9	48 ± 8	50 ± 6	48.3 ± 5	47 ± 5	47 ± 6	48 ± 4	45 ± 6
Week 3	193 ± 19	175 ± 16	179 ± 19	171* ±13	137 ± 12	134 ± 11	139 ± 12	135 ± 12
Week 4	238 ± 25	214 ± 22	221 ± 23	209* ± 15	153 ± 12	152 ± 13	157 ± 9.3	155 ± 16
Week 5	284 ± 28	252* ± 26	262 ± 24	252* ± 20	172 ± 11	168 ± 15	173 ± 13	173 ± 17
Weight gain								
Week 0-1	40 ± 4	37 ± 5	35 ± 6	33* ± 5	34 ± 4	34 ± 3	33 ± 4	32 ± 5
Week 2-3	49 ± 5	44 ± 5	46 ± 5	42** ± 5	24 ± 4	21 ± 8	21 ± 6	23 ± 5
Week 0-5	231 ± 24	204* ± 21	212 ± 20	203* ± 17	125 ± 9	121 ± 12	125 ± 12	128 ± 15
Subset IV								
Body weight								
Week 0	48 ± 5	49 ± 5	49 ± 5	45 ± 6	48 ± 7	42 ± 6	47 ± 6	45 ± 6
Week 5	257 ± 19	259 ± 13	265 ± 17	237 ± 36	171 ± 15	168 ± 9	173 ± 16	169 ± 9
Weight gain								
Week 0-1	37 ± 5	38 ± 3	38 ± 6	31* ± 6	35 ± 4	33 ± 4	32 ± 6	32 ± 4
Week 0-5	209 ± 16	210 ± 10	216 ± 13	192 ± 32	123 ± 11	126 ± 9	126 ± 12	123 ± 8
Subset V								
Body weight								
Week 0	51± 5	49 ± 7	50 ± 5	46 ± 3	46 ± 5	45 ± 5	50 ± 4	47 ± 7
Week 1	90 ± 8	89 ± 11	88 ± 11	78* ± 4	79 ± 8	78 ± 8	82 ± 6	78 ± 11
Weight gain								
Week 0-1	39 ± 5	40 ± 5	38 ± 7	32* ± 3	34 ± 4	33 ± 4	32 ± 4	30 ± 5
Subset VI								
Body weight								
Week 0	49 ± 6	48 ± 6	51 ± 4	46 ± 4	48 ± 6	45 ± 6	48 ± 8	46 ± 6
Week 6	292 ± 22	288 ± 20	298 ± 21	284 ± 19	176 ± 13	175 ± 12	190 ± 18	182 ± 12
Weight gain								
Week 0-6	243 ± 19	240 ± 18	247 ± 18	238 ± 16	127 ± 11	130 ± 10	142* ± 13	136 ± 9

[a] Data obtained from pages 132- 147, MRID 46210605.

[b] The first week after weaning is designated as week 0, the second week as week 1, etc.; data rounded to nearest whole number by reviewer.

* Statistically different from control, $p<0.05$

** Statistically different from control, $p<0.01$

3. Developmental landmarks:

a. Sexual maturation: The time to sexual maturation was generally comparable between treated and control male and female offspring, with the exception that male offspring from the mid-dose group had a statistically decreased time to preputial separation. Despite the decreases in mean pup weight at the time of sexual maturation in male and female offspring in the high-dose group, their time to sexual maturation was not affected. The data are presented in Table 7.

TABLE 7. Mean (∀SD) age of sexual maturation (days) [a]				
Parameter	Dose (mg/kg)			
	0	7.5	15	30
N (M/F)	29/30	30/30	30/30	29/30
Preputial separation (males)	44.4 ± 1.8	43.7 ± 1.5	43.2* ± 1.2	45.3 ± 2.4
Body weight at criterion (males)	188.3 ± 14.4	180.5 ± 10.5	181.5 ± 14.6	176.7* ± 14.5 (-6) [b]
Vaginal opening (females)	32.3 ± 2.1	32.2 ± 1.5	31.9 ± 2.0	32.1 ± 2.0
Body weight at criterion (females)	98.9 ± 13.9	94.4 ± 10.4	96.4 ± 10.0	89.5* ± 8.3 (-10)

[a] Data obtained from pages 122-123, MRID 46210605.

[b] Number in parentheses is the percent increase or decrease relative to controls; calculated by reviewer

* Statistically different from control, $p<0.05$

b. **Physical landmarks**: Physical landmarks, such as age of eye opening or incisor eruption, were not recorded.

4. Behavioral assessments:

a. **Open field observation:** Exposure-related open field observations for offspring in Subset IV are summarized in Table 8. High-dose F_1 males exhibited a slight increase in sudden movements or "jerky" standing on PNDs 45 and 60. While high-dose F_1 females did not exhibit the same increase in activity, an increase in the number of animals exhibiting tremors was evidenced on PND 4, with an increase in the severity of tremors noted on PND 11. No other clearly defined effects of treatment were observed.

TABLE 8. Functional observational battery results (incidence) [a]				
Observation	Dose (mg/kg)			
	0	7.5	15	30
Males				
Activity/Arousal level : Slightly increased, sudden movements or jerky standing:				
-PND 4	0	0	0	0
-PND 11	0	0	0	0
-PND 21	0	0	0	0
-PND 35	0	1	0	2
-PND 45	0	1	1	3
-PND 60	0	1	0	5
Females				
Tremors:				
-PND 4	1 (1) [b]	1 (1)	3 (1)	4 (1)
-PND 11	1 (1)	4 (1)	3 (1.3)	4 (1.5)
-PND 21	0	0	0	0
-PND 35	0	0	0	0
-PND 45	0	0	0	0
-PND 60	0	0	0	0

[a] Data obtained from pages 172-219, MRID 46210605.

[b] Number in parenthesis is the severity ranking: 0 = no tremors; 1 = slight tremors; 2 = moderate tremors; 3 = marked tremors

N = 10/sex/dose

b. Motor activity: Motor activity as assessed by total distance measured in cm or by the number of rearings is presented in Tables 9a and 9b, respectively. No consistent treatment-related effects were observed until PND 60, when statistically significant increases in mean total distance were present in male and female offspring from the high-dose group, and in female offspring from the mid-dose group, and the mean number of rearings was statistically increased in the high-dose females. Examination of subsession data of total distance on PND 60 revealed that male offspring from the high-dose group had statistically significantly increased values during subsessions 1-6, and females during subsessions 1-9 (see Table 10a). The female offspring from the mid-dose group showed a statistically significant increase only during subsession 5. PND 60 subsession data of the number of rearings revealed that offspring from the high-dose group had statistically significantly increases during subsessions 2-9 in females, and during subsessions 2-4 in males.

Development of habituation was seen as early as PND 13 in female offspring, with clear evidence of habituation in both male and female offspring by PND 17.

TABLE 9a. Mean (∀S.D.) motor activity data (total distance moved in cm for session ± SD) [a]

Test Day (Subset IV)	Dose (mg/kg)			
	0	7.5	15	30
Males				
PND 13	1658.9 ± 539.7	2209.6 ± 933.6	1979.6 ± 1314.7	1288.1 ± 488.3
PND 17	3285.9 ± 2147.0	3137.8 ± 2573.1	2307.9 ± 1730.3	1798.3 ± 685.5
PND 21	3705.5 ± 861.1	3967.5 ± 2208.3	3788.7 ± 1371.7	4451.7 ± 1717.6
PND 60	7761.9 ± 1271.9	7370.8 ± 1817.6	8155.4 ± 1701.1	11670** ± 2778.6
Females				
PND 13	1838.8 ± 1138.6	1802.8 ± 1007.9	1798.1 ± 997.1	1218.9 ± 505.4
PND 17	2761.4 ± 2839.5	2850.2 ± 2592.0	2587.6 ± 1958.9	2052.4 ± 1461.2
PND 21	3517.0 ± 873.6	4226.7 ± 1683.4	4565.8 ± 1680.0	4852.5 ± 1769.0
PND 60	8679.2 ± 1554.0	9694.0 ± 1677.4	10608* ± 1420.3	15795** ± 2886.0

[a] Data obtained from pages 220-235, MRID 46210605.
N = 4 - 10
* Statistically different from control, $p<0.05$
** Statistically different from control, $p<0.01$

TABLE 9b. Mean (∀S.D.) number of rearings (± SD) [a]				
Test Day (Subset IV)	Dose (mg/kg)			
	0	7.5	15	30
Males				
PND 13	38.9 ± 20.4	62.3 ± 41.8	65.3 ± 66.0	29.1 ± 26.7
PND 17	101.2 ± 80.1	110.9 ± 90.1	70.1 ± 53.7	46.5 ± 24.6
PND 21	79.3 ± 17.7	89.9 ± 75.8	67.4 ± 28.1	61.5 ± 25.3
PND 60	200.8 ± 56.8	227.5 ± 94.4	225.1 ± 47.9	229.5 ± 52.2
Females				
PND 13	68.3 ± 49.6	22.6 ± 28.6	46.6 ± 36.9	24.3 ± 22.7
PND 17	87.0 ± 92.5	81.1 ± 100.8	74.2 ± 45.8	47.7 ± 23.9
PND 21	80.8 ± 34.8	101.0 ± 30.7	90.6 ± 60.1	64.2 ± 56.5
PND 60	232.8 ± 62.7	259.1 ± 64.1	256.3 ± 38.7	360.7* ± 69.6

[a] Data obtained from pages 236-251, MRID 46210605.
N = 10 for males; 8-10 for females
* Statistically different from control, p<0.05

TABLE 10a. Motor activity sub-session data for PND 60 (mean distance moved in cm ∀S.D.) [a]					
Sub-session		Dose (mg/kg)			
		0	7.5	15	30
Males					
PND 60	1	1333.8 ± 131.8	1259.0 ± 207.3	1211.4 ± 157.1	1893.4** ± 389.9
	2	1094.5 ± 150.8	1096.9 ± 146.2	1087.1 ± 162.3	1762.9** ± 461.2
	3	907.5 ± 174.0	903.0 ± 151.3	927.4 ± 188.0	1511.2** ± 505.0
	4	788.3 ± 202.3	799.2 ± 276.8	853.0 ± 206.5	1497.3** ± 371.0
	5	660.9 ± 295.6	681.9 ± 159.5	729.8 ± 181.5	1066.0* ± 368.4
	6	686.5 ± 282.5	563.9 ± 337.7	690.6 ± 190.5	1138.2** ± 355.1
	7	556.3 ± 250.6	573.9 ± 256.4	736.6 ± 276.3	861.9 ± 396.9
	8	532.3 ± 243.2	499.6 ± 270.2	533.9 ± 243.2	580.9 ± 486.6
	9	342.0 ± 241.4	330.5 ± 259.4	565.2 ± 237.6	416.4 ± 332.3
	10	225.3 ± 276.6	292.7 ± 332.2	328.8 ± 336.3	283.9 ± 459.9
	11	280.8 ± 275.4	130.5 ± 164.4	267.3 ± 270.4	256.4 ± 420.4
	12	353.6 ± 268.5	239.8 ± 337.3	224.5 ± 293.6	401.1 ± 531.3
Females					
PND 60	1	1607.4 ± 128.2	1623.9 ± 223.0	1656.8 ± 373.8	2264.8** ± 222.8
	2	1221.2 ± 132.0	1198.2 ± 135.4	1331.8 ± 217.6	2055.2** ± 232.0
	3	999.9 ± 134.3	948.9 ± 133.7	1161.2 ± 209.3	1732.6** ± 403.3
	4	861.5 ± 152.8	898.0 ± 78.0	980.1 ± 313.6	1570.8** ± 431.6
	5	743.2 ± 168.8	817.1 ± 189.9	1009.3* ± 237.2	1479.7** ± 355.2
	6	672.0 ± 221.0	729.3 ± 256.5	765.3 ± 310.4	1463.0** ± 217.7
	7	534.0 ± 198.1	740.6 ± 407.6	787.2 ± 270.9	1161.0** ± 459.6
	8	541.8 ± 308.1	633.1 ± 312.0	677.1 ± 189.6	1150.0* ± 547.4
	9	498.9 ± 288.7	510.7 ± 224.5	699.1 ± 239.2	1010.0* ± 462.1
	10	502.9 ± 375.7	600.7 ± 435.3	528.7 ± 353.1	792.7 ± 337.5
	11	245.1 ± 239.0	553.0 ± 429.3	493.0 ± 268.6	662.9 ± 548.2
	12	251.3 ± 322.8	440.7 ± 313.7	518.9 ± 365.4	452.0 ± 638.5

[a] Data obtained from pages 226-227; 234-235, MRID 46210605.
N = 10 for males; 8-10 for females
* Statistically different from control, p<0.05
** Statistically different from control, p<0.01

TABLE 10b. Motor activity sub-session data for PND 60 (mean number of rearings ∀S.D.) [a]

Sub-session		Dose (mg/kg)			
		0	7.5	15	30
Males					
PND 60	1	38.5 ± 8.0	38.9 ± 10.0	40.7 ± 11.0	41.1 ± 10.3
	2	32.2 ± 5.4	35.2 ± 8.9	36.2* ± 5.8	44.4* ± 12.1
	3	25.2 ± 6.9	30.3 ± 8.5	29.0 ± 6.1	30.2* ± 12.1
	4	18.0 ± 3.6	23.7 ± 10.5	23.1 ± 6.4	34.8** ± 10.8
	5	16.1 ± 6.7	18.1 ± 6.6	19.6 ± 4.9	19.9 ± 6.1
	6	17.3 ± 8.2	14.9 ± 12.1	14.8 ± 6.1	19.9 ± 5.7
	7	12.1 ± 6.2	15.3 ± 11.3	16.8 ± 8.1	14.2 ± 8.8
	8	12.0 ± 7.5	15.0 ± 11.4	14.3 ± 10.3	9.3 ± 10.3
	9	9.1 ± 8.9	12.8 ± 12.0	13.8 ± 8.0	5.2 ± 5.9
	10	5.9 ± 9.6	10.7 ± 13.3	6.7± 8.7	3.2 ± 7.0
	11	7.2 ± 8.7	5.4 ± 9.2	5.8 ± 7.0	2.2 ± 3.7
	12	7.2 ± 7.3	7.2 ± 10.1	4.3 ± 6.9	5.1 ± 8.2
Females					
PND 60	1	46.1 ± 8.7	48.6 ± 10.6	48.9 ± 10.4	53.1 ± 8.3
	2	36.6 ± 7.5	37.4 ± 4.2	35.6 ± 8.6	51.4** ± 8.3
	3	29.8 ± 8.3	27.8 ± 8.0	28.7 ± 6.6	45.0** ± 9.2
	4	25.6 ± 9.8	27.5 ± 7.9	27.0 ± 6.1	40.6** ± 8.9
	5	20.3 ± 7.1	21.9 ± 6.5	23.0 ± 7.9	32.7 ± 12.9
	6	18.3 ± 8.5	17.0 ± 5.8	17.3 ± 7.6	28.7 ± 12.3
	7	14.4 ± 8.3	18.9 ± 13.7	18.3 ± 7.2	23.3 ± 8.6
	8	12.4 ± 9.5	14.4 ± 9.1	14.7 ± 5.2	27.1* ± 12.9
	9	9.9 ± 6.0	13.5 ± 9.4	12.7 ± 4.9	23.8** ± 11.1
	10	10.6 ± 9.4	12.5 ± 10.9	12.2 ± 8.4	15.6 ± 8.3
	11	3.9 ± 5.0	10.8 ± 9.7	8.3 ± 5.4	10.4 ± 10.0
	12	4.9 ± 10.6	9.0 ± 7.3	9.6 ± 7.2	9.0 ± 13.7

[a] Data obtained from pages 242-243; 250-251, MRID 46210605.
N = 10 for males and 8-10 for females
* Statistically different from control, $p<0.05$
** Statistically different from control, $p<0.01$

c. Auditory startle reflex habituation:

The mean overall (block 1-5) peak amplitude and mean latency to peak data for males and females (Subset III) are presented in Table 11a, while the mean for individual blocks in males and females are presented in Tables 11b and 11c, respectively. The only statistically significant difference was in the mean peak amplitude on PND 60 in F_1 females from the high-dose group. Examination of individual block data revealed increases in the mean peak amplitude on PND 60 in F_1 females from the high-dose group for blocks 2-5, with the mean for blocks 2, 4, and 5 attaining statistical significance. Although the mean peak amplitude on PND 60 in the F_1 mid-dose females was statistically increased only in block 2, the mean peak amplitude for all of the blocks thereafter continued to be increased to at least 31% above the control values.

TABLE 11a. Mean (∀SD) overall (Blocks 1-5) acoustic startle peak amplitude (g) and latency to peak (msec) (Subset III) [a]

Dose (mg/kg)	Parameter	Males		Females	
		PND 24	PND 60	PND 24	PND 60
0	Peak Amp.	377.0 ± 144.2	1025.1 ± 678.4	283.6 ± 81.6	415.2 ± 179.7
	Latency	30.5 ± 5.4	34.9 ± 6.6	31.5 ± 5.0	31.8 ± 5.6
7.5	Peak Amp.	393.1 ± 111.4	902.0 ± 534.8	307.5 ± 70.0	427.7 ± 185.7
	Latency	31.9 ±4.3	39.1 ± 9.9	31.6 ± 3.9	33.4 ± 5.9
15	Peak Amp.	381.6 ± 99.2	595.7 ± 317.3	290.0 ± 74.3	579.4 ± 202.3 (+40) [b]
	Latency	34.3 ± 4.3	31.2 ± 4.1	30.2 ± 4.9	30.3 ± 5.1
30	Peak Amp.	324.1 ± 62.3	883.0 ± 529.9	279.6 ± 75.9	617.5* ± 206.1 (+49)
	Latency	32.6 ± 8.0	32.6 ± 10.7	32.5 ± 8.3	30.5 ± 6.6

[a] Data were obtained from Study Report Tables 1A 147- 1A 151; pages 252-259, MRID 46210605; n=9-10.

[b] Number in parentheses is the percent increase or decrease relative to controls; calculated by reviewer

* Significantly different from controls at p#0.05

TABLE 11b. Mean (±SD) interval acoustic startle peak amplitude (g) and latency to peak (msec) in F_1 male rats[a]

Dose (mg/kg)	Parameter	Block 1	Block 2	Block 3	Block 4	Block 5
PND 24						
0	Peak Amp.	392.2 ± 135.1	371.5 ± 155.1	348.6 ± 169.9	366.7 ± 146.8	406.2 ± 180.8
	Latency	34.6 ± 3.4	30.3 ± 8.0	28.2 ± 6.0	29.6 ± 9.6	29.9 ±8.7
7.5	Peak Amp.	388.1 ± 197.0	405.4 ± 157.9	381.5 ± 148.9	404.9 ± 145.9	385.5 ± 165.0
	Latency	33.9 ± 7.3	34.4 ± 4.1	30.5 ± 6.1	31.1 ± 6.8	29.5 ± 7.0
15	Peak Amp.	386.5 ± 106.4	366.5 ± 118.8	425.3 ± 166.7	377.3 ± 148.7	352.3 ± 134.0
	Latency	39.8 ± 7.1	34.8 ± 4.3	32.8 ± 6.9	32.4 ± 6.8	32.0 ± 3.9
30	Peak Amp.	331.5 ± 63.2	307.8 ± 74.4	359.3 ± 127.1	292.3 ± 66.5	329.8 ± 59.8
	Latency	38.5 ± 15.5	33.6 ± 18.4	32.4 ± 6.7	28.2 ± 4.8	30.1 ± 5.8
PND 60						
0	Peak Amp.	1368.3 ± 496.2	1007.6 ± 809.3	981.5 ± 840.4	925.5 ± 862.2	842.6 ± 719.9
	Latency	43.2 ± 10.3	33.9 ± 8.0	36.4 ± 11.5	29.8 ± 7.6	31.1 ± 6.8
7.5	Peak Amp.	1305.7 ± 769.2	940.2 ± 637.5	903.4 ± 773.2	730.7 ± 574.7	629.9 ± 353.9
	Latency	47.5 ± 19.4	41.8 ± 14.8	37.6 ± 14.4	36.1 ± 9.6	32.7 ± 9.0
15	Peak Amp.	906.7 ± 295.5	578.8 ± 313.7	513.7 ± 372.9	465.3 ± 292.5	513.7 ± 401.0
	Latency	35.7 ± 5.9	32.4 ± 8.8	30.7 ± 7.2	27.9 ± 5.3	29.1 ± 4.6
30	Peak Amp.	1416.3 ± 940.1	922.8 ± 548.7	783.7 ± 618.0	622.4 ± 345.1	669.8 ± 394.7
	Latency	48.6 ± 20.9	30.7 ± 10.6	26.4 ± 9.6	28.6 ± 8.5	29.0 ± 7.9

[a] Data were obtained from pages 252-253; 256-257, MRID 46210605.

TABLE 11c. Mean (±SD) interval acoustic startle peak amplitude (g) and latency to peak (msec) in F_1 female rats[a]

Dose (mg/kg)	Parameter	Block 1	Block 2	Block 3	Block 4	Block 5
PND 24						
0	Peak Amp.	325.8 ± 101.9	294.4 ± 106.9	266.3 ± 99.1	245.3 ± 71.0	286.4 ± 92.2
	Latency	35.8 ± 10.0	36.1 ± 11.6	27.3 ± 4.4	28.6 ± 4.4	29.8 ± 5.2
7.5	Peak Amp.	348.1 ± 79.1	282.1 ± 55.2	313.4 ± 91.6	310.9 ± 93.8	283.1 ± 89.0
	Latency	35.0 ± 7.7	30.9 ± 8.3	32.2 ± 6.7	30.6 ± 5.5	29.4 ± 4.4
15	Peak Amp.	294.7 ± 102.2	275.4 ± 75.8	268.8 ± 73.6	303.4 ± 102.5	308.0 ± 111.4
	Latency	36.5 ± 4.6	32.0 ± 9.4	28.3 ± 6.9	27.1 ± 5.9	26.8 ± 5.0
30	Peak Amp.	289.8 ± 88.4	291.5 ± 111.9	288.3 ± 85.5	268.9 ± 79.8	259.6 ± 55.3
	Latency	41.2 ± 16.7	33.4 ± 12.5	29.4 ± 8.2	29.0 ± 8.1	29.3 ± 7.2
PND 60						
0	Peak Amp.	596.1 ± 376.7	416.4 ± 268.2	383.1 ± 152.3	371.0 ± 159.6	309.3 ± 114.7
	Latency	36.1 ± 10.9	31.4 ± 8.6	29.9 ± 5.7	30.0 ± 5.8	31.5 ± 5.7
7.5	Peak Amp.	632.6 ± 316.9	421.7 ± 188.1	390.7 ± 193.9	334.2 ± 186.1	359.2 ± 166.5
	Latency	37.0 ± 8.2	33.0 ± 6.3	34.1 ± 6.1	33.1 ± 9.8	29.8 ± 7.0
15	Peak Amp.	870.4 ± 450.3	571.4** ± 145.4 (+37)[b]	501.9 ± 177.1 (+31)	485.0 ± 197.8 (+31)	468.1 ± 251.8 (+51)
	Latency	36.0 ± 9.5	31.7 ± 6.6	28.6 ± 5.0	27.9 ± 6.0	27.4 ± 6.1
30	Peak Amp.	706.0 ± 348.7	679.8* ± 366.2 (+63)	637.6 ± 289.5 (+66)	535.5* ± 133.6 (+44)	528.6** ± 127.1 (+71)
	Latency	37.6 ± 11.2	33.0 ± 10.4	27.5 ± 5.8	25.9 ± 6.3	28.7 ± 8.7

[a] Data were obtained from pp. 254-255; 258-259, MRID 46210605.

[b] Percent difference from controls (calculated by reviewers) is presented parenthetically

* Significantly different from controls at p#0.05

** Significantly different from controls at p#0.01

d. Learning and memory testing: The number and percentage of animals reaching the criteria (finding a ladder on the correct side of the water maze pool) (Subset V and VI) are presented in Table 12. In male offspring on PND 60, a statistically significant decrease in the number of animals completing the test within the designated time period was noted at the high-dose during trial six in the first learning block and in the memory test one week later. In female offspring on PND 60, a dose-related decrease in the number of animals completing the test within the designated time period occurred during the second learning block during trials 2-5.

TABLE 12. Water maze performance - Males (number (%) reaching criteria) [a]

Test		Dose (mg/kg)			
		0	7.5	15	30
PND 23 (Subset V)					
Learning 1	Trial 2	3 (30)	4 (40)	6 (60)	4 (40)
	Trial 3	7 (70)	5 (50)	6 (60)	4 (40)
	Trial 4	8 (80)	6 (60)	7 (70)	6 (60)
	Trial 5	7 (70)	8 (80)	8 (80)	8 (80)
	Trial 6	9 (90)	6 (60)	7 (70)	6 (60)
Memory		7 (70)	5 (50)	8 (80)	7 (70)
Learning 2	Trial 2	1 (10)	1 (10)	1 (10)	1 (10)
	Trial 3	4 (40)	3 (30)	3 (30)	2 (20)
	Trial 4	5 (50)	4 (40)	5 (50)	4 (40)
	Trial 5	4 (40)	6 (60)	5 (50)	5 (50)
	Trial 6	6 (60)	7 (70)	6 (60)	4 (40)
PND 60 (Subset VI)					
Learning 1	Trial 2	4 (40)	6 (60)	5 (50)	6 (67)
	Trial 3	6 (60)	6 (60)	7 (70)	3 (33)
	Trial 4	7 (70)	7 (70)	7 (70)	3 (33)
	Trial 5	9 (90)	7 (70)	7 (70)	5 (56)
	Trial 6	9 (90)	9 (90)	8 (80)	4* (44)
Memory		9 (90)	9 (0)	8 (80)	3* (33)
Learning 2	Trial 2	2 (20)	2 (20)	3 (30)	3 (33)
	Trial 3	7 (70)	4 (40)	2* (20)	4 (44)
	Trial 4	8 (80)	4 (40)	4 (40)	6 (67)
	Trial 5	6 (60)	4 (40)	5 (50)	4 (44)
	Trial 6	7 (70)	5 (50)	7 (70)	7 (78)

[a] Data obtained from pages 148 and 150, MRID 46210605.

N = 9,10

* Statistically different from control, $p<0.05$

** Statistically different from control, $p<0.01$

TABLE 13. Water maze performance – Females (number (%) reaching criteria) [a]

Test		Dose (mg/kg)			
		0	7.5	15	30
PND 23					
Learning 1	Trial 2	3 (30)	5 (50)	4 (40)	5 (50)
	Trial 3	6 (60)	5 (50)	8 (80)	4 (40)
	Trial 4	8 (80)	7 (70)	5 (50)	3* (30)
	Trial 5	7 (70)	4 (40)	7 (70)	7 (70)
	Trial 6	7 (70)	6 (60)	8 (80)	8 (80)
Memory		5 (50)	5 (50)	6 (60)	4 (40)
Learning 2	Trial 2	1 (10)	2 (20)	1 (10)	2 (20)
	Trial 3	4 (40)	6 (60)	3 (30)	2 (20)
	Trial 4	7 (70)	5 (50)	3 (30)	2* (20)
	Trial 5	5 (50)	6 (60)	5 (50)	3 (30)
	Trial 6	4 (40)	7 (70)	3 (30)	5 (50)
PND 60					
Learning 1	Trial 2	3 (30)	3 (30)	6 (60)	5 (50)
	Trial 3	5 (50)	3 (30)	7 (70)	5 (50)
	Trial 4	6 (60)	4 (40)	4 (40)	8 (80)
	Trial 5	6 (60)	4 (40)	7 (70)	7 (70)
	Trial 6	8 (80)	2* (20)	6 (60)	5 (50)
Memory		6 (60)	6 (60)	8 (80)	7 (70)
Learning 2	Trial 2	4 (40)	3 (30)	1 (10)	0* (0)
	Trial 3	6 (60)	4 (40)	1* (10)	0** (0)
	Trial 4	7 (70)	4 (40)	2* (20)	1** (10)
	Trial 5	8 (80)	4 (40)	4 (40)	3* (30)
	Trial 6	6 (60)	2 (20)	4 (40)	5 (50)

[a] Data obtained from pages149 and 151, MRID 46210605.
N = 10
* Statistically different from control, $p<0.05$
** Statistically different from control, $p<0.01$

5. Postmortem results:

a. Brain weight: Mean brain weight data are presented in Table 14. Mean absolute brain weight was decreased in the offspring from the mid- and high-dose groups at all timepoints. Brain-to-body weight ratios generally followed a dose-related, statistically significant decrease in the mid- and high-dose F_1 males and females on PNDs 11, 22, and 62. The only statistically significant difference noted in mean terminal body weight occurred in F_1 high-dose males on PND 62.

TABLE 14. Mean (∀SD) brain weight data [a]

Parameter	Dose (mg/kg)			
	0	7.5	15	30
Males				
PND 11				
Terminal body weight (g)	21.73 ± 2.09	21.49 ± 2.42	23.16 ± 1.67	20.01 ± 1.70
Brain weight (g)	1.15 ± 0.08	1.15 ± 0.07	1.10 ± 0.06	0.85** ± 0.04 (-26)[b]
Brain-to-body weight ratio	5.34 ± 0.52	5.37 ± 0.43	4.78* ± 0.41 (-10)	4.24** ± 0.26 (-21)
PND 22				
Terminal body weight (g)	48.29 ± 2.68	47.78 ± 4.23	50.47 ± 4.71	43.98 ± 4.19
Brain weight (g)	1.67 ± 0.04	1.66 ± 0.06	1.56** ± 0.05 (-7)	1.20** ± 0.06 (-28)
Brain-to-body weight ratio	3.47 ± 0.15	3.50 ± 0.32	3.12* ± 0.33 (-10)	2.75** ± 0.29 (-21)
Termination – PND 62				
Terminal body weight (g)	300.03 ± 29.35	272.14 ± 24.60	280.19 ± 24.39	263.09** ± 18.31 (-12)
Brain weight (g)	2.03 ± 0.07	1.92* ± 0.12 (-5)	1.81** ± 0.10 (-11)	1.50** ± 0.081 (-26)
Brain-to-body weight ratio	0.68 ± 0.06	0.71 ± 0.03	0.65 ± 0.05	0.57** ± 0.04 (-16)
Females				
PND 11				
Terminal body weight (g)	21.93 ± 1.98	20.60 ± 5.21	21.32 ± 2.12	18.92 ± 3.09
Brain weight (g)	1.15 ± 0.05	1.08 ± 0.18	1.02** ± 0.042 (-11)	0.82** ± 0.07 (-29)
Brain-to-body weight ratio	5.28 ± 0.39	5.42 ± 0.85	4.82* ± 0.40 (-9)	4.40** ± 0.42 (-17)
PND 22				
Terminal body weight (g)	48.71 ± 4.51	46.83 ± 2.83	46.19 ± 4.02	42.66 ± 4.87
Brain weight (g)	1.64 ± 0.05	1.58* ± 0.05 (-4)	1.39** ± 0.05 (-15)	1.19** ± 0.06 (-27)
Brain-to-body weight ratio	3.40 ± 0.29	3.39 ± 0.20	3.04* ± 0.27 (-11)	2.82** ± 0.32 (-17)
Termination – PND 62				
Terminal body weight (g)	179.33 ± 13.52	174.92 ± 14.84	180.31 ± 10.12	180.36 ± 17.62
Brain weight (g)	1.80 ± 0.07	1.84 ± 0.07	1.66** ± 0.10 (-8)	1.43** ± 0.04 (-21)
Brain-to-body weight ratio	1.01 ± 0.06	1.06 ± 0.09	0.92* ± 0.08 (-9)	0.80** ± 0.06 (-21)

[a] Data obtained from pages 284-294, MRID 46210605.

[b] Number of parentheses is the percent increase or decrease relative to controls; calculated by reviewer

N =9-10

* Statistically different from control, $p<0.05$

** Statistically different from control, $p<0.01$

b. Neuropathology:

1) **Macroscopic examination:** Macroscopic examination of selected rats revealed an increased incidence of hypoplasia of the cortex cerebri in the brain of F1 rats from the mid- and high-dose groups on PNDs 11, 22, and 62 (see Table 15). No other gross lesions were reported.

TABLE 15. Macroscopic findings in F_1 rats: Incidence of hypoplasia of cortex cerebri (number animals affected/total number of animals examined) [a]

Test Day	Dose (mg/kg)			
	0	7.5	15	30
Males				
PND 11	0/10	0/10	10/10	10/10
PND 22	0/10	0/10	1/10	7/10
PND 62	0/10	1/10	10/10	10/10
Females				
PND 13	0/10	0/10	10/10	10/10
PND 21	0/10	0/10	4/10	10/10
PND 60	0/10	0/10	9/10	10/10

[a] Data obtained from pages 296-298, MRID 46210605.

2) **Microscopic examination:** A summary of treatment-related microscopic findings is presented in Table 16. Microscopic examination of F_1 rats on PND 11, 22, and 62 revealed hypoplasia in the frontal lobe and cortical hypoplasia and ectopic hippocampi neurons in the parietal lobe. These findings were present in mid- and/or high-dose males and females. On PND 62, ventricle dilation in the parietal lobe was also noted in all the high-dose males and females. Axonal degeneration was present in the distal tibial nerve of one F_1 high-dose male and in the proximal sciatic nerve in one high-dose female.

TABLE 16. Histopathology findings (number of animals affected) [a]

Parameter	Dose (mg/kg)			
	0	7.5	15	30
Males				
PND 11				
Frontal lobe:				
Cortical hypoplasia	0	0	10	10
Parietal lobe:				
Cortical hypoplasia	0	0	10	10
Ectopic hipp. neurons	0	0	0	8
PND 22				
Frontal lobe:				
Cortical hypoplasia	0	0	1	7
Parietal lobe:				
Cortical hypoplasia	0	0	0	10
Ectopic hipp. neurons	0	0	0	8
Termination (PND 62)				
Frontal lobe:				
Cortical hypoplasia	0	0	10	10
Parietal lobe:				
Cortical hypoplasia	0	0	10	10
Ectopic hipp. neurons	0	0	0	7
Ventricle dilation	0	0	0	10
Females				
PND 11				
Frontal lobe:				
Cortical hypoplasia	0	0	10	10
Parietal lobe:				
Cortical hypoplasia	0	0	10	10
Ectopic hipp. neurons	0	0	0	8
PND 22				
Frontal lobe:				
Cortical hypoplasia	0	0	4	10
Parietal lobe:				
Cortical hypoplasia	0	0	0	10
Ectopic hipp. neurons	0	0	0	6
Termination (PND 62)				
Frontal lobe:				
Cortical hypoplasia	0	0	9	10
Parietal lobe:				
Cortical hypoplasia	0	0	10	10
Ectopic hipp. neurons	0	0	0	9
Ventricle dilation	0	0	0	10

[a] Data obtained from pages 299-301, MRID 46210605.
n = 10 except for the male control group on PND 62 where n = 9

Morphometric evaluation of offspring on PND 11, 22, and 62 revealed dose-related decreases in brain length and width, left and right frontal cortex, left and right parietal cortex, left and right nucleus caudatus, corpus callosum, left and right hippocampus, and folium pyramis (half) in both sexes. Data are summarized in Tables 17-18.

TABLE 17. Mean (∀SD) morphometric data: Males [a]

Parameter (µm unless otherwise noted)	Dose (mg/kg)			
	0	**7.5**	**15**	**30**
PND 11				
Brain: Length (cm)	1.617 ± 0.044	1.613 ± 0.031	1.609 ± 0.053	1.453** ± 0.025 (-10) [b]
Width (cm)	1.336 ± 0.028	1.333 ± 0.031	1.330 ± 0.027	1.233** ± 0.019 (-8)
Frontal cortex: Left	1280 ± 93	1465 ± 118	1318 ± 196	971** ± 149 (-24)
Right	1281 ± 97	1465 ± 133	1311 ± 156	980** ± 160 (-23)
Parietal cortex: Left	1362 ± 138	1465 ± 114	1278 ± 174	985** ± 42 (-28)
Right	1321 ± 121	1452 ± 131	1296 ± 147	997** ± 85 (-25)
Nucleus caudatus: Left	2648 ± 172	2674 ± 101	2694 ± 237	2300** ± 318 (-13)
Right	2558 ± 158	2658 ± 89	2573 ± 255	2147** ± 231 (-16)
Corpus callosum	348 ± 61	376 ± 65	336 ± 145	207** ± 30 (-41)
Hippocampus: Left	1292 ± 154	1370 ± 133	1260 ± 178	894** ± 106 (-31)
Right	1271 ± 173	1318 ± 174	1271 ± 144	877** ± 128 (-31)
PND 22				
Brain: Length (cm)	1.858 ± 0.037	1.843 ± 0.025	1.815** ± 0.020 (-2)	1.673** ± 0.048 (-10)
Width (cm)	1.502 ± 0.015	1.482 ± 0.022	1.453** ± 0.015 (-3)	1.324** ± 0.012 (-12)
Frontal cortex: Left	1574 ± 94	1590 ± 123	1488 ± 215	1268** ± 156 (-19)
Right	1597 ± 119	1589 ± 98	1523 ± 204	1268** ± 115 (-21)
Parietal cortex: Left	1613 ± 124	1694 ± 106	1531 ± 122	1281** ± 99 (-21)
Right	1658 ± 109	1669 ± 112	1531* ± 115	1280** ± 101 (-23)
Nucleus caudatus: Left	3402 ± 175	3493 ± 176	3447 ± 287	2994** ± 239 (-12)
Right	3296 ± 150	3400 ± 165	3374 ± 97	3015** ± 178 (-9)
Corpus callosum	342 ± 70	367 ± 63	334 ± 58	217** ± 89 (-37)
Hippocampus: Left	1649 ± 119	1599 ± 85	1449** ± 133 (-12)	1120** ± 100 (-32)
Right	1655 ± 132	1588 ± 77	1452** ± 90 (-12)	1121** ± 109 (-32)
Folium pyramis half	326 ± 35	314 ± 23	300* ± 22 (-8)	303* ± 22 (-7)
Termination (PND 62)				
Brain: Length (cm)	2.062 ± 0.041	2.016* ± 0.052 (-2)	1.988** ± 0.033 (-4)	1.849** ± 0.040 (-10)
Width (cm)	1.540 ± 0.036	1.510* ± 0.042 (-2)	1.489* ± 0.037 (-3)	1.384** ± 0.019 (-10)
Frontal cortex: Left	1748 ± 138	1735 ± 107	1616* ± 69 (-8)	1282** ± 93 (-27)
Right	1776 ± 131	1768 ± 97	1649* ± 87 (-7)	1323** ± 138 (-26)
Parietal cortex: Left	1842 ± 103	1803 ± 129	1623** ± 91 (-12)	1349** ± 113 (-27)
Right	1813 ± 159	1769 ± 134	1573** ± 81 (-13)	1293** ± 115 (-29)
Nucleus caudatus: Left	4000 ± 187	3938 ± 215	3878 ± 237	3435** ± 236 (-14)
Right	3791 ± 187	3772 ± 304	3777 ± 258	3348** ± 279 (-12)
Corpus callosum	455 ± 105	405 ± 68	374* ± 54 (-18)	220** ± 75 (-52)
Hippocampus: Left	1755 ± 105	1776 ± 105	1646* ± 97 (-6)	1310** ± 166 (-25)
Right	1832 ± 119	1786 ± 114	1674** ± 116 (-9)	1324** ± 142 (-28)
Folium pyramis half	394 ± 29	366 ± 30	348** ± 19 (-12)	360** ± 36 (-9)

[a] Data obtained from pages 302-307; 308-309; 312-313; and 316-317, MRID 46210605.

[b] Number of parentheses is the percent increase or decrease relative to controls; calculated by reviewer

N = 9-10

* Statistically different from control, $p<0.05$

** Statistically different from control, $p<0.01$

TABLE 18. Mean (∀SD) morphometric data: Females [a]

Parameter (µm unless otherwise noted)	**Dose (mg/kg)**			
	0	**7.5**	**15**	**30**
PND 11				
Brain: Length (cm)	1.608 ± 0.025	1.592 ± 0.104	1.571* ± 0.040 (-2) [b]	1.452** ± 0.037 (-10)
Width (cm)	1.354 ± 0.018	1.320 ± 0.077	1.295** ± 0.023 (-4)	1.221** ± 0.042 (-10)
Frontal cortex: Left	1386 ± 93	1403 ± 158	1181** ± 154 (-15)	1077** ± 136 (-22)
Right	1373 ± 122	1415 ± 145	1225* ± 177 (-11)	1066** ± 135 (-22)
Parietal cortex: Left	1425 ± 124	1389 ± 176	1194** ± 149 (-16)	1149** ± 147 (-19)
Right	1346 ± 97	1430 ± 152	1228 ± 187	1076** ± 138 (-20)
Nucleus caudatus: Left	2885 ± 176	2635** ± 266	2562** ± 144 (-11)	2316** ± 292 (-20)
Right	2768 ± 175	2634 ± 314	2540** ± 115 (-8)	2239** ± 266 (-19)
Corpus callosum:	478 ± 157	360* ± 83 (-25)	290** ± 50 (-39)	190** ± 57 (-60)
Hippocampus: Left	1310 ± 151	1271 ± 180	1213 ± 99	848** ± 93 (-35)
Right	1313 ± 132	1264 ± 160	1208* ± 93	874** ± 120 (-33)
PND 22				
Brain: Length (cm)	1.837 ± 0.033	1.823 ± 0.020	1.745** ± 0.023 (-5)	1.683** ± 0.032 (-8)
Width (cm)	1.477 ± 0.018	1.462 ± 0.020	1.389** ± 0.015 (-6)	1.326** ± 0.028 (-10)
Frontal cortex: Left	1574 ± 121	1582 ± 91	1412** ± 125 (-10)	1221** ± 141 (-22)
Right	1573 ± 89	1642 ± 138	1478* ± 144 (-6)	1266** ± 150 (-20)
Parietal cortex: Left	1654 ± 106	1655 ± 116	1480** ± 121 (-11)	1300** ± 129 (-21)
Right	1619 ± 72	1640 ± 133	1410** ± 94 (-13)	1281** ± 121 (-21)
Nucleus caudatus: Left	3284 ± 169	3612 ± 185	3293 ± 203	3084** ± 206 (-6)
Right	3198 ± 152	3370 ± 242	3245 ± 161	3026* ± 163 (-5)
Corpus callosum:	377 ± 103	359 ± 54	321 ± 50	162** ± 39 (-57)
Hippocampus: Left	1676 ± 89	1577* ± 79 (-6)	1409** ± 123 (-16)	1092** ± 109 (-35)
Right	1651 ± 79	1561* ± 109 (-5)	1402** ± 112 (-15)	1112** ± 95 (-33)
Termination (PND 62)				
Brain: Length (cm)	1.982 ± 0.044	1.986 ± 0.033	1.950 ± 0.034	1.830** ± 0.021 (-8)
Width (cm)	1.479 ± 0.019	1.496 ± 0.032	1.432** ± 0.041 (-3)	1.353** ± 0.035 (-9)
Frontal cortex: Left	1693 ± 74	1692 ± 52	1568* ± 148 (-7)	1215** ± 110 (-28)
Right	1726 ± 77	1728 ± 77	1582** ± 118 (-8)	1259** ± 85 (-27)
Parietal cortex: Left	1726 ± 124	1733 ± 86	1551** ± 126 (-10)	1298** ± 97 (-25)
Right	1682 ± 107	1652 ± 58	1504** ± 145 (-11)	1299** ± 48 (-23)
Nucleus caudatus: Left	3828 ± 167	3891 ± 174	3830 ± 221	3396** ± 269 (-11)
Right	3746 ± 186	3817 ± 110	3575* ± 206 (-5)	3349** ± 182 (-11)
Corpus callosum:	427 ± 80	449 ± 93	370 ± 64	209** ± 51 (-51)
Hippocampus: Left	1671 ± 103	1776 ± 92	1637 ± 101	1275** ± 116 (-24)
Right	1721 ± 129	1773 ± 94	1674 ± 89	1267** ± 116 (-26)

[a] Data obtained from pages 302-307; 310-311; 314-315; and 318-319, MRID 46210605.

[b] Number of parentheses is the percent increase or decrease relative to controls; calculated by reviewer

N = 9-10

* Statistically different from control, $p<0.05$

** Statistically different from control, $p<0.01$

III. DISCUSSION and CONCLUSIONS:

A. **INVESTIGATORS= CONCLUSIONS**: Single administration of 30 and 15 mg/kg of methylazoxy methanol acetate on GD 15 resulted in abnormal and retarded development of a variety of brain structures in offspring which was evidenced as:

- gross lesions (hypoplasia of cortex, cerebri)
- neurohistopathological lesions (only at 30 mg/kg; disorganization of the cortical layers, clusters of ectopic hippocampal and periventricular neurons, ventricle dilation (only on PND 62))
- weight decreases (absolute and relative brain weights)
- reduced values in diverse morphometric brain measurements. The main reduced values were found for the corpus callosum, the hippocampus, the parietal and the frontal cortices, in that order. Basal ganglia (nucleus caudatus/putamen) and the cerebellum (folium pyramis, external germinal matrix layer) also showed reduced size as well, indicating a treatment-related influence on the development of the brain.

Some indications of a treatment-related effect were also found in the 7.5 mg/kg dose group, mainly as changes in morphometric parameters. A clear dose-dependent effect was detected for many, but not all, neuropathological parameters.

Morphological alterations of the developing nervous system were associated with clinical observations of neurotoxicity at dose levels of 30 and 15 mg/kg. Clear signs of neurotoxicity, such as increased (motor) activity level, impaired auditory startle habituation, and retarded learning/relearning capability, were found in the high- and mid-dose offspring, even though the physical development of these animals was only slightly or not at all affected by the test compound. The clinical neurotoxicity effects were slightly more apparent in females. It was obvious that morphological alterations of the developing nervous system did not become clinically evident in most cases until the affected offspring had developed to young adults (around PND 60).

The results of this study demonstrate the ability of this laboratory to detect the developmental neurotoxicity induced by MAM.

B. REVIEWER COMMENTS: In this positive control study, MAM was used to demonstrate neurotoxicity in offspring following maternal treatment. Dose-related alterations were found in the following parameters: open field observation, motor activity assessments, auditory startle reflex habituation (females), learning and memory testing (water maze performance), brain weight, gross and microscopic pathological findings in the brain, and morphometric measurements of areas of the brain. Treatment of dams at the high-dose also produced a clear reduction in mean pre-weaning pup weight and weight gain and inconsistent reductions in post-weaning body weight/body weight gain. The laboratory was able to demonstrate the ability to detect a dose-response in developmental neurotoxic effects.

The Experimental Toxicology and Ecology Facility of the BASF Corporation has demonstrated proficiency in testing for developmental neurotoxicity in rats through open field observation, motor activity assessment, auditory startle reflex habituation, learning and memory testing (water maze performance), brain weight, gross and microscopic pathological findings in the brain, and morphometric brain measurements.

C. STUDY DEFICIENCIES:

Study deficiencies include:

- analytical determination of the stability, homogeneity and concentration of the test formulations was not performed
- some details about the conduct of the open field observation were not provided (such as whether the same technicians were used throughout testing, if the technicians were blind to treatment status of animals, description of the environmental conditions, and duration of testing).

The deviations did not affect the acceptability of the study because the study was designed as a positive control study.